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(54) Title: COMPOUNDS FOR STIMULATING P-GLYCOPROTEIN FUNCTION AND USES THEREOF

(57) Abstract: The present invention is directed to polypeptides (e.g., fragments) derived from P-glycoprotein and caveolin-1 which are capable of inhibiting the interaction between these two proteins. Inhibition of this interaction leads to increase of efflux of compounds that are transported by P-gp. The invention further includes methods of treating patients having diseases that benefit from increased P-gp-mediated efflux. Such diseases include neoplasms such as cancer and neurological diseases such as neurodegenerative diseases.

COMPOUNDS FOR STIMULATING P-GLYCOPROTEIN FUNCTION AND USES THEREOF

Background of the Invention

The invention relates to the field of therapeutic compounds, their uses, methods of use and compositions comprising them. This invention also relates to the treatment of diseases resulting from accumulation of P-glycoprotein substrates. This invention also relates to the treatment of diseases associated with cellular migration and/or angiogenesis and neurological diseases.

ABC (ATP-Binding Cassette) transporters superfamily members are expressed on most mammalian tissues with excretory and/or barrier function. These transporters are involved in unidirectional substrate translocation and use ATP as the energy source to activate the extrusion process. ABC transporters appear to have developed as a mechanism to protect the body from harmful substances.

P-glycoprotein (P-gp) is an ABC transporter, product of the *MDR1* gene, found in the liver, gut, gonads, kidneys, biliary system, brain, and other organs. P-glycoprotein is an efflux pump protecting the structural and functional integrity of the organs and tissues on which it is expressed. P-glycoprotein is localized at the plasma membrane, more specifically in microdomains enriched in cholesterol called caveolae. Caveolae may act as signaling platforms and can be identified by the presence of specific markers such as caveolin-1, -2, and -3. This particular localization seems to be important for P-gp ATPase and transport activities. Recent work suggests that two P-gp populations co-exist in the plasma membrane surrounded by different cholesterol concentration in the P-gp closed microenvironment (Barakat et al., *Biochem. J.* 388:563-571, 2005). P-glycoprotein is associated with multi-drug resistance. Indeed, P-gp interacts with a wide variety of anti-cancer drugs leading to a decrease in their

intracellular concentrations ultimately leading to failure of chemotherapy. P-glycoproteins have also been linked to neurological diseases. Indeed, neurological disorders including but not limited to epilepsy, Alzheimer's disease and Huntington's disease are associated with overexpression of ABC efflux transporters or substrates. Apart from their efflux transport activity, P-glycoproteins are also known to play a role in cellular migration and angiogenesis.

Given the involvement of P-gp substrates in disease, there is a need to develop therapeutic approaches aimed at regulating P-gp function, which can reduce the accumulation of P-gp substrates, can inhibit cellular migration or angiogenesis, or can treat neurological disorders and other diseases.

Summary of the Invention

In a first aspect, the invention features a polypeptide including an amino acid sequence having at least 50% (e.g., at least 60%, 70%, 80%, 90%, 95%, 99%, or 100%) identity to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR or a peptide described in Table 1, where the polypeptide is less than 170 (e.g., less than 150, 125, 100, 75, 50, 40, 30, 25, 20, 15) amino acids in length. The polypeptide may be capable of interacting with (e.g., specifically binding) P-glycoprotein. In certain embodiments, a polypeptide having sequence identity to DGIWKASFTTFTVTKYWFYR has a valine or leucine at the position corresponding to the thirteenth amino acid, has a tyrosine at the position corresponding to the sixteenth amino acid, has a lysine or arginine at the position corresponding to the twentieth amino acid, or any combination thereof. In other embodiments where the polypeptide has sequence identity to VTKYWFYR, the polypeptide has a valine or leucine at the position corresponding to the first amino acid, has a tyrosine at the position corresponding to the fourth amino acid, has a lysine or arginine at the position

corresponding to the eighth amino acid, or any combination thereof. In some embodiments, the polypeptide is soluble or the polypeptide is substantially pure. The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

The invention also features a polypeptide including (a) an amino acid sequence having at least 50% (e.g., at least 60%, 70%, 80%, 90%, 95%, 99%, or 100%) identity to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR, and (b) a heterologous sequence (e.g., any sequence described herein). The polypeptide may be capable of interacting with (e.g., specifically binding) P-glycoprotein. In some embodiments, the polypeptide is soluble or the polypeptide is substantially pure. The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

In another aspect, the invention features a soluble polypeptide including a fragment of caveolin-1 (e.g., any fragment described herein), where the fragment binds (e.g., specifically binds) to a portion of P-glycoprotein (e.g., amino acids 36-47 of P-glycoprotein). The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

In another aspect, the invention features a polypeptide including an amino acid sequence having at least 50% (e.g., at least 60%, 70%, 80%, 90%, 95%, 99%, or 100%) identity to the sequence VFSMFRYSNWLDK or a peptide described in Table 2, where the peptide is less than 1000 (e.g., less than 750, 500, 250, 200, 150, 100, 90, 75, 60, 50, 40, 35, 30, 25, or 20) amino acids in length. The polypeptide may be capable of interacting with (e.g., specifically binding) caveolin-1. In certain embodiments, the peptide has a valine or leucine at the position corresponding to the first amino acid, has a tyrosine at the position corresponding to the seventh amino acid, has a lysine or arginine at the position corresponding to the thirteenth amino acid, or a combination

thereof. In some embodiments, the polypeptide is soluble or the polypeptide is substantially pure. The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

In another aspect, the invention features a polypeptide including (a) an amino acid sequence having at least 50% (e.g., at least 60%, 70%, 80%, 90%, 95%, 99%, or 100%) identity to the sequence VFSMFRYSNWLDK, and (b) a heterologous sequence (e.g., any sequence described herein). The polypeptide may be capable of interacting with (e.g., specifically binding) caveolin-1. In some embodiments, the polypeptide is soluble or the polypeptide is substantially pure. The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

In another aspect, the invention features a soluble polypeptide including a fragment of P-glycoprotein, where the fragment binds to a portion of caveolin-1 (e.g., amino acids 82-101). In some embodiments, the polypeptide is soluble or the polypeptide is substantially pure. The invention also features a composition including the polypeptide and a pharmaceutically acceptable carrier.

In any of the above aspects, the polypeptide may differ from the sequence corresponding to either wild-type human caveolin-1 or human P-gp by at least a single amino substitution or deletion (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions or deletions). In any of the above aspects, the polypeptide may include unnatural amino acid substitutions (e.g., those described herein) and may include peptidomimetics, such as those described herein. In certain embodiments, the polypeptide having a substitution or deletion may have increased (e.g., at least 5%, 10%, 25%, 50%, 75%, 100%, 200%, 500%, 1000%, 5000%, 10,000%, 50,000%) activity (e.g., P-gp efflux activity, stronger binding to either caveolin-1 or to P-gp). In certain embodiments, the polypeptide having a substitution or deletion exhibits

decreased angiogenesis, or decreased or decreased cellular migration (e.g., at least 5%, 10%, 25%, 50%, 75%, 80%, 90%, 95%, or 99%) as compared to a polypeptide having the corresponding wild-type sequence.

In another aspect, the invention features a method of increasing P-gp-mediated efflux in a cell (e.g., in a patient), the method including administering a polypeptide of any of the previous aspects in an amount sufficient to increase P-gp-mediated efflux. In certain embodiments, the cell is in a patient. The patent may be suffering from a neoplasm (e.g., cancer) or from a neurological disease and the polypeptide may be administered in an amount sufficient to treat the neoplasm or the neurological disease. The neoplasm may be a cancer selected from the group consisting of leukemia, polycythemia vera, lymphoma, Waldenstrom's macroglobulinemia, heavy chain disease, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, glioblastoma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma. The neurological disease may be a neurodegenerative disease, which in turn, may be Alzheimer's disease,

Parkinson's disease, Huntington's disease, prion disease, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, Lewy body disease, or Pick's disease. In other embodiments, the patient is suffering from a disease or condition selected from the group consisting of drug intoxications (e.g., overdose), inclusion body myositis, cerebral amyloid angiopathy, amyloidosis (e.g., AA-type), and eye diseases (e.g., macular degeneration and glaucoma).

By "fragment" is meant a portion of a full length polypeptide sequence of at least 4, 5, 6, 7, 8, 10, 15, 20, 25, 40, 50, 60, 75, 90, 100, 125, 150, 200, 250, 300, 500, 750, or 1000 amino acids.

By "specifically binds" or "specific binding" is meant a compound (e.g., a polypeptide) or antibody that recognizes and binds a desired polypeptide but that does not substantially recognize and bind other molecules in a sample, for example, a biological sample. Binding may occur with a dissociation constant of at least 1000 μmol, 100 μmol, 10 μmol, 1 μmol, 100 nm, 10 nm, 1 nm, 100 pmol, 10 pmol, or 1 pmol.

By "substantially pure" or is meant a nucleic acid, polypeptide, or other molecule that has been separated from the components that naturally accompany it. Typically, a polypeptide is substantially pure when it is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. For example, a substantially pure polypeptide may be obtained by extraction from a natural source, by expression of a recombinant nucleic acid in a cell that does not normally express that protein, or by chemical synthesis.

By a "decrease" in the level of expression or activity of a gene or protein is meant a reduction in protein or nucleic acid level or activity in a cell, a cell extract, or a cell supernatant. For example, such a decrease may be due to

inhibition by an exogenous compound RNA stability, transcription, or translation, increased protein degradation, or RNA interference. Preferably, this decrease is at least 5%, 10%, 25%, 50%, 75%, 80%, or even 90% of the level of expression or activity under control conditions. The decrease may be assayed by measuring changes in phenotypic response. In some embodiments, the decrease may be identified by measuring changes in angiogenesis or cell migration using, for example, the assays described herein.

By an "increase" in the expression or activity of a gene or protein is meant a positive change in protein or nucleic acid level or activity in a cell, a cell extract, or a cell supernatant. For example, such a increase may be due to competitive inhibition of an inhibitor, increased RNA stability, transcription, or translation, or decreased protein degradation. Preferably, this increase is at least 5%, 10%, 25%, 50%, 75%, 80%, 100%, 200%, or even 500% or more over the level of expression or activity under control conditions. The increase may be assayed by measuring changes in phenotypic response. In some embodiments, the increase may be identified by measuring changes in angiogenesis or cell migration using, for example, the assays described herein.

By "patient" is meant either a human or non-human animal (e.g., a mammal).

"Treating" a disease or condition in a subject or "treating" a patient having a disease or condition refers to subjecting the individual to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease or condition is decreased, stabilized, or prevented.

By "soluble" is meant soluble in aqueous solution under physiological conditions.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figures 1A and **1B** are graphs showing decreases in vinblastine accumulation in cells upon treatment with an examplary pepPgp peptide (VFSMFRYSNWLDK) or an exemplary pepCav peptide (DGIWKASFTTFTVTKYWFYR) over time (Figure 1A) or using increasing pepPgp or pepCav concentrations (Figure 1B).

Figure 2 is a graph showing decrease of TAXOL entering the brain upon treatment either with pepCav or with pepPgp.

Figure 3A is a set of photomicrographs showing decreased cellular migration upon administration of pepCav or pepPgp to cells.

Figure 3B is a pair of graphs showing decreased migration of MDCK cells that overexpress *MDR1* upon administration of pepPgp or pepCav.

Figure 4A is a photomicrograph and graph showing decreased tube (capillary) formation upon administration pepCav and pepPgp to HUVEC cells. This administration reduced the capillary-like structure formation by 75% and 40%, respectively.

Figure 4B is a photomicrograph and a graph showing decreased tube using the Matrigel plug assay. The graph shows measured hemoglobin content in the plug, a measure of capillary formation. PepCav and PepPgp reduced hemoglobin content by 25% and 40%, respectively as compared to the controls (bFGF alone).

Figure 5 depicts the amino acid sequences of human caveolin-1 and human P-glycoprotein, with the binding regions shown in bold.

Detailed Description

P-glycoprotein is a membrane protein capable of transporting many different molecules from a cell. Caveolin-1, another membrane protein, is

capable of interacting with P-gp and inhibits P-gp efflux activity. We, for the first time, have identified peptides derived from each of caveolin-1 and P-gp (e.g., pepCav and pepPgp) that are capable of blocking the caveolin-1/P-gp interaction (e.g., through competitive inhibition) and increasing the efflux activity of P-gp. Accordingly, the polypeptides of the invention may be useful in treatment of diseases (e.g., neurological diseases and neoplasms such as those described herein) where increased P-gp efflux is desired. In addition, we have shown that administration of these peptides decreases cellular migration and angiogenesis, thus indicating use of these peptides in treating neoplasms such as cancer.

Caveolin-1

Caveolin-1 is a 178 amino acid member of the caveolin family of proteins, which includes caveolin-1, -2, and -3. These proteins are typically found the caveolae, which are membrane invaginations found in many cell types. The caveolae are involved in endocytosis. Caveolin-1 interacts with many proteins (see, e.g., Table 2 of Razani et al., *Pharmacol. Rev.* 54:431-467, 2002, the entirety of which is hereby incorporated by reference) including P-gp (Jodoin et al., *J. Neurochem.* 87:1010-1023, 2003). Caveolin-1 is also involved in membrane transport, lipid, trafficking, and signal transduction. Caveolin-1 also forms homo-oligomers in cells. Caveolin-1 contains a scaffolding domain (amino acids residues 82-101), which is capable of binding P-gp (Demeule et al., *Vascul. Pharmacol.* 38:339-348, 2002). The scaffolding domain of caveolin-1 is involved in interactions with numerous proteins and was shown to negatively regulate some signaling molecules localized in caveolae, including eNOS, protein kinase C, and epidermal growth factor receptor (Okamoto et al., *J. Biol. Chem.* 273:5419-5422, 1998). In contrast to this, interaction of caveolin-1 with the insulin receptor increases insulin-stimulated

phosphorylation of downstream targets (Yamamoto et al., *J. Biol. Chem.* 273:26962-26968, 1998).

P-glycoprotein

P-glycoprotein (P-gp) is a 1280 amino acid member of the ATP binding cassette (ABC) transporters and is capable of transporting compounds from cells. P-gp is encoded by the *MDR1* gene; expression of this gene has been associated with multi-drug resistant cancer. Indeed P-gp is capable of transporting a variety of agents out of cells, including chemotherapeutic agents.

P-glycoprotein substrates may be endogenous and/or exogenous substances. These substrates encompass anticancer agents, immunosuppressive agents, HIV protease inhibitors, bioactive polypeptides, cardiac drugs, toxic peptides, and cytokines. Specific examples of P-gp substrates include beta-amyloid, taxol, taxol derivatives, cyclosporine A, vinblastine, vincristine, etoposide, doxorubicin, cyclophosphamide, taxotere, melphalan, chlorambucil, pharmaceutically acceptable salts and combination thereof as well as anticancer drugs such as vinca alkaloids, epipodophyllotoxins, anthracyclines, and taxanes that may be P-glycoprotein substrates among others as described by Turcotte et al., "The Blood-Brain Barrier: Roles of the Multidrug Resistance Transporter P-glycoprotein. Chapter 19 in Blood-Brain Interfaces: From Ontogeny to Artificial Interfaces. edited by R. Dermietzel, D. C. Spray and M. Nedergaard, Wiley-VCH, Weinheim, pp. 431-461, 2006.

P-gp activity has been linked to a number of disease states including neoplasms and neurological disorders. Here, we show that inhibition of P-gp activity results in decreased angiogenesis and cellular migration (see, e.g., the examples herein). In addition, polymorphisms in the P-gp gene have been associated with Parkinson's disease (PD) (Furuno et al., *Pharmacogenetics*

2:529-534, 2002). While P-gp expression is correlated with drug resistance in cancer cells, P-gp expression is down-regulated in neurological diseases, including Creutzfeldt-Jakob disease (CJD) (Vogelgesang et al., *Acta. Neuropathol. (Berl.)* 111:436-443, 2006). Further evidence of P-gp involvement in neurological diseases is shown by its ability to transport the amyloid-β peptide out of the brain (Lam et al., *J. Neurochem.* 76:1121-1128, 2001). Lowered expression or activity of P-gp at the blood-brain barrier may therefore be correlated with development of neurological diseases, and increasing P-gp activity may be useful to treat neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, prion disease, bovine spongiform encephalopathy (BSE), CJD, Lewy body disease, and Pick's disease.

P-gp contains a consensus caveolin-binding motif that binds to the scaffolding domain of caveolin. Three related caveolin-binding motifs include ΦXΦXXXXΦ, ΦXXXXΦXXΦ and ΦXΦXXXXΦXXΦ, where Φ is a phenylalanine, tyrosine, or tryptophan and X is any amino acid.

Consensus binding domains

An exemplary sequence derived from the cholesterol binding sequence localized on caveolin-1, DGIWKASFTTFT**V**TK**Y**WFY**R**. An exemplary sequence derived from the P-gp protein is **V**FSMFR**Y**SNWLD**K**, the P-gp cholesterol binding sequence. Both of these exemplary sequences conform to the cholesterol binding consensus motif:

L/V-A2-Y-A3-K/R

where each of A2 and A3 independently represents between one and five amino acids. The amino acids may be any naturally occurring amino acids (e.g., alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

proline, serine, threonine, tryptophan, tyrosine, and valine) or any modified or non-naturally occurring amino acids (e.g., those described herein).

Accordingly, peptides of the invention can have the formula:

A1-L/V-A2-Y-A3-K/R-A4

and may be capable of stimulating P-glycoprotein efflux, transport activity, or may inhibit cellular migration and/or angiogenesis. A1 may be absent or an amino acid sequence of 1 to about 19 amino acids; A2 is an amino acid sequence consisting of 1 to 5 amino acids; A3 is an amino acid sequence consisting of 1 to 5 amino acids and A4 may be absent or an amino acid sequence of 1 up to about 19 amino acids. Each amino acid may be any amino acid (e.g., any of those described herein).

Caveolin-1 fragments

We have identified the exemplary fragments DGIWKASFTTFTVTKYWFYR and VTKYWFYR derived from the caveolin-1 sequence as being capable of enhancing P-gp efflux activity. On this basis, the invention provides polypeptides including these fragments. The polypeptides of the invention may include an amino acid sequence with at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or 100% identity to a fragment of the caveolin-1 sequence. Preferred polypeptides are soluble under physiological conditions or can bind to P-glycoprotein and increase its efflux activity. Exemplary fragments are shown in Table 1. In certain embodiments, the polypeptide includes the fragment DGIWKASFTTFTVTKYWFYR or VTKYWFYR, or portions thereof (see e.g., Table 1). Polypeptides of the invention may also include additional portions of the caveolin-1 sequence (e.g., at least 1, 2, 3, 4, 6, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 100 additional amino acids at the N-terminal of the fragment, the C-terminal of the fragment, or both). Polypeptides of the invention can also include heterologous

sequences (e.g., as described herein). In some embodiments, the polypeptides of the invention may include tandem repeats (e.g., at least 2, 3, 5, 8, 10, or 15 repeats) of a P-gp fragment.

Table 1: Exemplary caveolin-1 fragments

DGIWKASFTTFTVTKYWFYR	ASFTTFTVTKYW	VTKYWFYR	WKASFT
GIWKASFTTFTVTKYWFYR	KASFTTFTVTKY	TVTKYWFY	IWKASF
DGIWKASFTTFTVTKYWFY	WKASFTTFTVTK	FTVTKYWF	GIWKAS
IWKASFTTFTVTKYWFYR	IWKASFTTFTVT	TFTVTKYW	DGIWKA
GIWKASFTTFTVTKYWFY	GIWKASFTTFTV	TTFTVTKY	YWFYR
DGIWKASFTTFTVTKYWF	DGIWKASFTTFT	FTTFTVTK	KYWFY
WKASFTTFTVTKYWFYR	TFTVTKYWFYR	SFTTFTVT	TKYWF
IWKASFTTFTVTKYWFY	TTFTVTKYWFY	ASFTTFTV	VTKYW
GIWKASFTTFTVTKYWF	FTTFTVTKYWF	KASFTTFT	TVTKY
DGIWKASFTTFTVTKYW	SFTTFTVTKYW	WKASFTTF	FTVTK
KASFTTFTVTKYWFYR	ASFTTFTVTKY	IWKASFTT	TFTVT
WKASFTTFTVTKYWFY	KASFTTFTVTK	GIWKASFT	TTFTV
IWKASFTTFTVTKYWF	WKASFTTFTVT	DGIWKASF	FTTFT
GIWKASFTTFTVTKYW	IWKASFTTFTV	TKYWFYR	SFTTF
DGIWKASFTTFTVTKY	GIWKASFTTFT	VTKYWFY	ASFTT
ASFTTFTVTKYWFYR	DGIWKASFTTF	TVTKYWF	KASFT
KASFTTFTVTKYWFY	FTVTKYWFYR	FTVTKYW	WKASF
WKASFTTFTVTKYWF	TFTVTKYWFY	TFTVTKY	IWKAS
IWKASFTTFTVTKYW	TTFTVTKYWF	TTFTVTK	GIWKA
GIWKASFTTFTVTKY	FTTFTVTKYW	FTTFTVT	DGIWK
DGIWKASFTTFTVTK	SFTTFTVTKY	SFTTFTV	WFYR
SFTTFTVTKYWFYR	ASFTTFTVTK	ASFTTFT	YWFY
ASFTTFTVTKYWFY	KASFTTFTVT	KASFTTF	KYWF
KASFTTFTVTKYWF	WKASFTTFTV	WKASFTT	TKYW
WKASFTTFTVTKYW	IWKASFTTFT	IWKASFT	VTKY
IWKASFTTFTVTKY	GIWKASFTTF	GIWKASF	TVTK
GIWKASFTTFTVTK	DGIWKASFTT	DGIWKAS	FTVT
DGIWKASFTTFTVT	TVTKYWFYR	KYWFYR	TFTV
FTTFTVTKYWFYR	FTVTKYWFY	TKYWFY	TTFT
SFTTFTVTKYWFY	TFTVTKYWF	VTKYWF	FTTF
ASFTTFTVTKYWF	TTFTVTKYW	TVTKYW	SFTT
KASFTTFTVTKYW	FTTFTVTKY	FTVTKY	ASFT
WKASFTTFTVTKY	SFTTFTVTK	TFTVTK	KASF
IWKASFTTFTVTK	ASFTTFTVT	TTFTVT	WKAS
GIWKASFTTFTVT	KASFTTFTV	FTTFTV	IWKA
DGIWKASFTTFTV	WKASFTTFT	SFTTFT	GIWK
TTFTVTKYWFYR	IWKASFTTF	ASFTTF	DGIW
FTTFTVTKYWFY	GIWKASFTT	KASFTT	
SFTTFTVTKYWF	DGIWKASFT		

Deletion analysis can be used to determine the minimal peptide sequence required for the interaction with P-gp or required to increase P-gp-mediated efflux. Deletion fragments (e.g., with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids deleted from the N-terminal, from the C-terminal, or from a combination thereof) of the DGIWKASFTTFTVTKYWFYR fragment can be generated using molecular biological methods known in the art and assayed for binding to P-gp or for increases in P-gp activity (see, e.g., the methods described below).

P-glycoprotein fragments

We have identified the exemplary fragment VFSMFRYSNWLDK derived from the P-gp sequence (amino acids 36-48) as binding caveolin-1 and capable of enhancing P-gp efflux activity. On this basis, the invention provides polypeptides including these fragments. The polypeptides of the invention may include an amino acid sequence with at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 99%, or 100% identity to a fragment (e.g., the fragments described herein) of the P-gp sequence. Preferred polypeptides are soluble under physiological conditions, can bind to caveolin-1, or can increase P-gp efflux activity. Exemplary fragments are shown in Table 2. In certain embodiments, the polypeptide includes the fragment VFSMFRYSNWLDK, or portions thereof (see e.g., Table 2). Polypeptides of the invention may also include additional portions of the P-gp sequence (e.g., at least 1, 2, 3, 4, 6, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 100, 200, 300, 400, 500, or 600 additional amino acids at the N-terminal of the fragment, the C-terminal of the fragment, or both). Polypeptides of the invention can also include heterologous sequences (e.g., as described herein). In some embodiments, the polypeptides of the invention may include tandem repeats (e.g., at least 2, 3, 5, 8, 10, or 15 repeats) of a P-gp fragment.

Table 2: P-glycoprotein fragments

VFSMFRYSNWLDK	FSMFRYSN	SNWLD
FSMFRYSNWLDK	VFSMFRYS	YSNWL
VFSMFRYSNWLD	YSNWLDK	RYSNW
SMFRYSNWLDK	RYSNWLD	FRYSN
FSMFRYSNWLD	FRYSNWL	MFRYS
VFSMFRYSNWL	MFRYSNW	SMFRY
MFRYSNWLDK	SMFRYSN	FSMFR
SMFRYSNWLD	FSMFRYS	VFSMF
FSMFRYSNWL	VFSMFRY	WLDK
VFSMFRYSNW	SNWLDK	NWLD
FRYSNWLDK	YSNWLD	SNWL
MFRYSNWLD	RYSNWL	YSNW
SMFRYSNWL	FRYSNW	RYSN
FSMFRYSNW	MFRYSN	FRYS
VFSMFRYSN	SMFRYS	MFRY
RYSNWLDK	FSMFRY	SMFR
FRYSNWLD	VFSMFR	FSMF
MFRYSNWL	NWLDK	VFSM
SMFRYSNW		

As with the caveolin-1 fragments, deletion analysis can also be used to determine the minimal peptide sequence required for the interaction of the P-gp fragments with caveolin-1 or the minimal peptide sequence required to increase P-gp-mediated efflux (e.g., using the assays described herein). Deletion fragments (e.g., with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids deleted from the N-terminal, from the C-terminal, or a combination thereof) of the VFSMFRYSNWLDK fragment can be generated using molecular biologic methods known in the art and assayed for binding to caveolin-1 or assayed for increases in P-gp activity (see, e.g., the methods described below).

Fusion proteins

The polypeptides of the invention include fusion proteins (e.g., a fragment of caveolin-1 or P-gp and a heterologous sequence). The caveolin-1 or P-gp portion of the fusion protein may be soluble (e.g., lacking the

transmembrane domains) or may be any functional fragment of caveolin-1 or P-gp (e.g., a fragment capable of regulating efflux activity of P-gp). The caveolin-1 or P-gp portion of the fusion protein may be at least 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to at least a portion (e.g., any portion described herein) of the full length caveolin-1 or P-gp protein (e.g., the human protein).

The caveolin-1 or P-gp fragment may be fused to one or more fusion partners at either its N-terminus or C-terminus. In certain embodiments, one of the fusion partners is the Fc protein (e.g., mouse Fc or human Fc). In other embodiments, the heterologous sequence may be a purification or detection tag, for example, proteins that may be detected directly or indirectly such as green fluorescent protein, hemagglutinin, or alkaline phosphatase), DNA binding domains (for example, GAL4 or LexA), gene activation domains (for example, GAL4 or VP16), purification tags, or secretion signal peptides (e.g., preprotyrypsin signal sequence). In other embodiments the fusion partner may be a tag, such as c-myc, poly histidine, or FLAG. Each fusion partner may contain one or more domains, e.g., a preprotrypsin signal sequence and FLAG tag.

The fusion proteins may include additional amino acid residues (e.g., at least 1, 2, 3, 4, 5, 8, 10, 12, 15, 20, 25, 40, 50, 75, 100) at either the N or C terminus (e.g., 1 to 10, 1 to 11, 1 to 12, 1 to 13, 1 to 14, 1 to 15, 1 to 16, 1 to 17 1 18, and 1 to 19 amino acids).

Peptide modifications

Peptides having a modification can be employed in the invention. Such modifications include may maintain or increase the biological activity of the original polypeptide or may optimize one or more of the particularity (e.g. stability, bioavailability, etc.) of the compounds of the invention. Polypeptides

of the invention may include for example, amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side-chains and the amino- or carboxy-terminus. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Other modifications include, for example, pegylation, acetylation, acylation, addition of acetomidomethyl (Acm) group, ADP-ribosylation, alkylation. amidation, biotinylation, carbamoylation, carboxyethylation, esterification, covalent attachment to flavin, covalent attachment to a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of drug, covalent attachment of a marker (e.g., fluorescent or radioactive), covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation and ubiquitination. In addition, polypeptides of the invention may include more than one modification.

Polypeptides of the invention may also include substitutions of non naturally occurring amino acids. A non-naturally occurring amino acid is to be

understood herein as an amino acid which is not naturally produced or found in a mammal. Non-naturally occurring amino acids include D-amino acids, amino acids having an acetylaminomethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid. Non-naturally occurring amino acids also include omega amino acids of the formula $NH_2(CH_2)_nCOOH$ where n is 2-6, sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, norleucine, and phenylglycine (e.g., a substitute for Trp, Tyr or Phe). Other amino acids include citrulline, methionine sulfoxide, cysteic acid, ornithine, hydroxyproline (e.g., in place of proline).

Preparation of Peptide Derivatives and Peptidomimetics

In addition to peptides consisting only of naturally occurring amino acids, peptidomimetics or peptide analogs are also encompassed by the present invention. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. The non-peptide compounds are termed "peptide mimetics" or peptidomimetics (Fauchere et al., *Infect. Immun.* 54:283-287 ,1986; Evans et al., *J. Med. Chem.* 30:1229-1239, 1987). Peptide mimetics that are structurally related to therapeutically useful peptides may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as —CH_2NH—, —CH_2S—, —CH_2—CH_2—, —CH=CH— (cis and trans), —CH_2SO—, —$CH(OH)CH_2$—, —$COCH_2$— etc., by methods well known in the art (Spatola, *Peptide Backbone Modifications, Vega Data,* 1(3):267, 1983); Spatola et al. (*Life Sci.* 38:1243-1249, 1986);

Hudson et al. (*Int. J. Pept. Res.* 14:177-185, 1979); and Weinstein. B., 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New-York). Such peptide mimetics may have significant advantages over naturally-occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency), reduced antigenicity and others.

While peptides are effective in stimulating P-gp-mediated efflux *in vitro*, their effectiveness *in vivo* might be reduced by the presence of proteases. Serum proteases have specific substrate requirements. The substrate must have both L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the protease activity in serum, usually act on the first peptide bond of the peptide and require a free N-terminus (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). In light of this, it is often advantageous to use modified versions of peptides. The modified peptides retain the structural characteristics of the original L-amino acid peptides that confer biological activity with regard to IGF-1, but are advantageously not readily susceptible to cleavage by protease and/or exopeptidases.

Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. Thus, a peptide derivative or peptidomimetic of the present invention may be all L, all D or mixed D, L peptide. The presence of an N-terminal or C-terminal D-amino acid increases the *in vivo* stability of a peptide since peptidases cannot utilize a D-amino acid as a substrate (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Reverse-D peptides are peptides containing D-amino acids, arranged in a reverse sequence relative to a peptide containing L-amino acids. Thus, the C-terminal residue of an L-amino acid peptide becomes N-terminal for the D-

amino acid peptide, and so forth. Reverse D-peptides retain the same tertiary conformation and therefore the same activity, as the L-amino acid peptides, but are more stable to enzymatic degradation *in vitro* and *in vivo*, and thus have greater therapeutic efficacy than the original peptide (Brady and Dodson, *Nature* 368:692-693, 1994; Jameson et al., *Nature* 368:744-746, 1994). In addition to reverse-D-peptide, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387-418, 1992). For example, constrained peptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic peptide. Cyclic peptides have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they are, of course, susceptible to endopeptidases, which do not cleave at peptide termini. The amino acid sequences of the peptides with N-terminal or C-terminal D-amino acids and of the cyclic peptides are usually identical to the sequences of the peptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

A cyclic derivative containing an intramolecular disulfide bond may be prepared by conventional solid phase synthesis while incorporating suitable S-protected cysteine or homocysteine residues at the positions selected for cyclization such as the amino and carboxy termini (Sah et al., *J. Pharm. Pharmacol.* 48:197, 1996). Following completion of the chain assembly, cyclization can be performed either (1) by selective removal of the S-protecting group with a consequent on-support oxidation of the corresponding two free SH-functions, to form a S-S bonds, followed by conventional removal of the product from the support and appropriate purification procedure or (2) by removal of the peptide from the support along with complete side chain de-

protection, followed by oxidation of the free SH-functions in highly dilute aqueous solution.

The cyclic derivative containing an intramolecular amide bond may be prepared by conventional solid phase synthesis while incorporating suitable amino and carboxyl side chain protected amino acid derivatives, at the position selected for cyclization. The cyclic derivatives containing intramolecular -S-alkyl bonds can be prepared by conventional solid phase chemistry while incorporating an amino acid residue with a suitable amino-protected side chain, and a suitable S-protected cysteine or homocysteine residue at the position selected for cyclization.

Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a peptide is to add chemical groups at the peptide termini, such that the modified peptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the peptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of peptides in human serum (Powell et al., *Pharm. Res.* 10:1268-1273, 1993). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular, the present invention includes modified peptides consisting of peptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

Also included by the present invention are other types of peptide derivatives containing additional chemical moieties not normally part of the peptide, provided that the derivative retains the desired functional activity of the peptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be

an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g., benzoyl) or a blocking group such as F-moc (fluorenylmethyl-O—CO—); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives conjugated to an antibody or other biological ligand and other types of derivatives.

Longer peptide sequences which result from the addition of additional amino acid residues to the peptides of the invention are also encompassed in the present invention. Such longer peptide sequence would be expected to have the same biological activity (e.g., binding to and stimulating P-gp-mediated efflux) as the peptides described above. While peptides having a substantial number of additional amino acids are not excluded, it is recognized that some large polypeptides may assume a configuration that masks the effective sequence, thereby preventing binding to the target (e.g., caveolin-1 or P-gp). These derivatives could act as competitive antagonists. Thus, while the present invention encompasses peptides or derivatives of the peptides described herein having an extension, desirably the extension does not destroy the P-gp efflux stimulatory activity of the peptide or derivative.

Other derivatives included in the present invention are dual peptides consisting of two of the same, or two different peptides of the present invention covalently linked to one another either directly or through a spacer, such as by a short stretch of alanine residues or by a putative site for proteolysis (e.g., by cathepsin, see e.g., U.S. Patent No. 5,126,249 and European Patent No. 495 049). Multimers of the peptides of the present invention consist of polymer of molecules formed from the same or different peptides or derivatives thereof.

The present invention also encompasses peptide derivatives that are chimeric or fusion proteins containing a peptide described herein, or fragment

thereof, linked at its amino- or carboxy-terminal end, or both, to an amino acid sequence of a different protein. Such a chimeric or fusion protein may be produced by recombinant expression of a nucleic acid encoding the protein. For example, a chimeric or fusion protein may contain at least 6 amino acids of a peptide of the present invention and desirably has a functional activity equivalent or greater than a peptide of the invention.

Peptide derivatives of the present invention can be made by altering the amino acid sequences by substitution, addition, or deletion or an amino acid residue to provide a functionally equivalent molecule, or functionally enhanced or diminished molecule, as desired. The derivative of the present invention include, but are not limited to, those containing, as primary amino acid sequence, all or part of the amino acid sequence of the peptides described herein (e.g., a VEGFR peptide 2.1, 2.2, or 2.3, or an APG-201, APG-202, APG-203, APG-204, APG-205, or APG-206 peptide, or an API-101, API-103, or API-106 peptide, or an API-401, API-402, API-403, API-404, or API-405 peptide) including altered sequences containing substitutions of functionally equivalent amino acid residues. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitution for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the positively charged (basic) amino acids include, arginine, lysine and histidine. The nonpolar (hydrophobic) amino acids include, leucine, isoleucine, alanine, phenylalanine, valine, proline, tryptophane and methionine. The uncharged polar amino acids include serine, threonine, cysteine, tyrosine, asparagine and glutamine. The negatively charged (acid) amino acids include glutamic acid and aspartic acid. The amino acid glycine may be included in either the nonpolar amino acid family or the uncharged (neutral) polar amino acid family.

Substitutions made within a family of amino acids are generally understood to be conservative substitutions.

Assays to Identify Peptidomimetics

As described above, non-peptidyl compounds generated to replicate the backbone geometry and pharmacophore display (peptidomimetics) of the peptides identified by the methods of the present invention often possess attributes of greater metabolic stability, higher potency, longer duration of action and better bioavailability.

The peptidomimetics compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, *Anticancer Drug Des.* 12:145, 1997). Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in: DeWitt et al. (*Proc. Natl. Acad. Sci. USA* 90:6909, 1993); Erb et al. (*Proc. Natl. Acad. Sci. USA* 91:11422, 1994); Zuckermann et al., *J. Med. Chem.* 37:2678, 1994); Cho et al. (*Science* 261:1303, 1993); Carell et al. (*Angew. Chem, Int. Ed. Engl.* 33:2059, 1994 and ibid 2061); and in Gallop et al. (*Med. Chem.* 37:1233, 1994). Libraries of compounds may be presented in solution (e.g., Houghten, *Biotechniques* 13:412-421, 1992) or on beads (Lam, *Nature* 354:82-84, 1991), chips (Fodor, *Nature* 364:555-556, 1993), bacteria or spores (U.S. Patent No. 5,223,409), plasmids (Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science* 249:386-390,

1990), or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

Once a peptide of the present invention is identified, it may be isolated and purified by any number of standard methods including, but not limited to, differential solubility (e.g., precipitation), centrifugation, chromatography (e.g., affinity, ion exchange, size exclusion, and the like) or by any other standard techniques used for the purification of peptides, peptidomimetics or proteins. The functional properties of an identified peptide of interest may be evaluated using any functional assay known in the art. Desirably, assays for evaluating downstream receptor function in intracellular signaling are used (e.g., cell proliferation).

For example, the peptidomimetics compounds of the present invention may be obtained using the following three-phase process: (1) scanning the peptides of the present invention to identify regions of secondary structure necessary for recognition and activity toward either caveolin-1 or P-gp; (2) using conformationally constrained dipeptide surrogates to refine the backbone geometry and provide organic platforms corresponding to these surrogates; and (3) using the best organic platforms to display organic pharmocophores in libraries of candidates designed to mimic the desired activity of the native peptide. In more detail the three phases are as follows. In phase 1, the lead candidate peptides are scanned and their structure abridged to identify the requirements for their activity. A series of peptide analogs of the original are synthesized. In phase 2, the best peptide analogs are investigated using the conformationally constrained dipeptide surrogates. Indolizidin-2-one, indolizidin-9-one and quinolizidinone amino acids (I^2aa, I^9aa and Qaa respectively) are used as platforms for studying backbone geometry of the best peptide candidates. These and related platforms (reviewed in Halab et al., *Biopolymers* 55:101-122, 2000; and Hanessian et al. *Tetrahedron* 53:12789-

12854, 1997) may be introduced at specific regions of the peptide to orient the pharmacophores in different directions. Biological evaluation of these analogs identifies improved lead peptides that mimic the geometric requirements for activity. In phase 3, the platforms from the most active lead peptides are used to display organic surrogates of the pharmacophores responsible for activity of the native peptide. The pharmacophores and scaffolds are combined in a parallel synthesis format. Derivation of peptides and the above phases can be accomplished by other means using methods known in the art.

Structure function relationships determined from the peptides, peptide derivatives, peptidomimetics or other small molecules of the present invention may be used to refine and prepare analogous molecular structures having similar or better properties. Accordingly, the compounds of the present invention also include molecules that share the structure, polarity, charge characteristics and side chain properties of the peptides described herein.

In summary, based on the disclosure herein, those skilled in the art can develop peptides and peptidomimetics screening assays which are useful for identifying compounds for interacting with and stimulating P-gp-mediated efflux. The assays of this invention may be developed for low-throughput, high-throughput, or ultra-high throughput screening formats. Assays of the present invention include assays which are amenable to automation.

Identification of additional P-gp inhibitory peptides

Additional peptides that inhibit P-gp efflux (e.g., derived from caveolin-1 or from P-gp) can be identified using the methods described herein or any other assays that can be used to measure P-gp activity known in the art. In certain embodiments, the test peptide is used in the cellular migration or the angiogenesis (e.g., in vivo or in vitro) assays described herein (see, e.g., Examples 4-6). In these embodiments, a test peptide is contacted with cells or

an organism expressing P-gp. Migration or blood vessel formation is measured in the presence or absence of these peptides. A decrease in either cellular migration or angiogenesis in the presence of the test peptide as compared to in the absence of the peptide identifies the test peptide as a P-gp-inhibitory peptide.

In other embodiments, efflux of a compound (e.g., vinblastine or TAXOL) in a cell expressing P-gp can be measured (see, e.g., Examples 1-3). Any compound known to be transported by P-gp can be used in these assays. The compound may be detectably labeled using any label known in the art (e.g., those described herein). In these exemplary methods, efflux of a compound is compared in the presence and absence of a test peptide, where increased efflux of the compound in the presence of the test peptide is indicative of the peptide having P-gp-stimulatory activity.

Other approaches, including in vitro binding assays, may be used to identify candidate peptides. In one particular embodiment, a test peptide that binds to P-gp or caveolin-1 or a portion thereof may be identified using a chromatography-based technique. For example, recombinant or synthetic fragments of either P-gp or caveolin-1 may be produced and purified by standard techniques and may be immobilized on a column. A test peptide or group of test peptides is then passed through the column, and a peptide specific for the bound P-gp or caveolin-1 fragment is identified on the basis of its ability to bind to the polypeptide and be immobilized on the column. To isolate the test peptide, the column is washed to remove non-specifically bound molecules, and the peptide of interest is then released from the column and collected. Peptides isolated by this method (or any other appropriate method) may, if desired, be further purified (e.g., by high performance liquid chromatography). Peptides isolated by this approach may also be used, for example, as therapeutics to treat a disorder in which P-gp efflux is decreased (e.g., any of

those described herein). Compounds which are identified as binding with an affinity constant less than or equal to 10 mM are considered particularly useful in the invention.

Nucleic acids

The present invention also includes nucleic acids encoding any of the polypeptides described herein. In some embodiments, the polynucleotide may be included in a vector suitable for expression of the polypeptide in a cell or in an organism (e.g., useful for gene therapy or protein expression as described below).

Polypeptide expression

In general, polypeptides of the invention may be produced by transformation of a suitable host cell with all or part of a polypeptide-encoding polynucleotide molecule or fragment thereof in a suitable expression vehicle.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant polypeptide. The precise host cell used is not critical to the invention. A polypeptide of the invention may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *Saccharomyces cerevisiae*, insect cells, e.g., Sf21 cells, or mammalian cells, e.g., NIH 3T3, HeLa, or preferably COS or CHO cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, Md.; also, see, e.g., Ausubel et al., supra). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors: A Laboratory Manual* (Pouwels, P. H. et al., 1985, Supp. 1987).

One particular bacterial expression system for polypeptide production is the *E. coli* pET expression system (Novagen, Inc., Madison, Wis.). According to this expression system, DNA encoding a polypeptide is inserted into a pET vector in an orientation designed to allow expression. Since the gene encoding such a polypeptide is under the control of the T7 regulatory signals, expression of the polypeptide is achieved by inducing the expression of T7 RNA polymerase in the host cell. This is typically achieved using host strains which express T7 RNA polymerase in response to IPTG induction. Once produced, recombinant polypeptide is then isolated according to standard methods known in the art, for example, those described herein.

Another bacterial expression system for polypeptide production is the pGEX expression system (Pharmacia). This system employs a GST gene fusion system which is designed for high-level expression of genes or gene fragments as fusion proteins with rapid purification and recovery of functional gene products. The polypeptide of interest is fused to the carboxyl terminus of the glutathione S-transferase protein from *Schistosoma japonicum* and is readily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B. Fusion proteins can be recovered under mild conditions by elution with glutathione. Cleavage of the glutathione S-transferase domain from the fusion protein is facilitated by the presence of recognition sites for site-specific proteases upstream of this domain. For example, polypeptides expressed in pGEX-2T plasmids may be cleaved with thrombin; those expressed in pGEX-3X may be cleaved with factor Xa.

Once isolated, the recombinant polypeptide can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

Polypeptides of the invention, particularly short peptide fragments, can also be produced by chemical synthesis (e.g., by the methods described in *Solid Phase Peptide Synthesis*, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein). In certain embodiments, a combination of techniques may be used to generate the fusion protein. For example, the protein and its fusion partner may be produced recombinantly and purified, or may be purified from a natural source, and then chemically coupled together to form the fusion protein.

Treatment methods of the invention

A polypeptide of the invention or polynucleotide encoding an polypeptide of the can be administered to a mammal (e.g., a human) suffering from any P-gp associated disorder. These disorders include any neoplasm such as a cancer, any neurological disorder such as a neurodegenerative disorder (e.g., those described herein), or any other disease described herein.

The polypeptide or polynucleotide may be administered by any route known in the art or described herein, for example, oral, parenteral (e.g., intravenously or intramuscularly), intraperitoneal, rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular. The polypeptide or polynucleotide may be administered in any dose or dosing regimen (e.g., those described herein).

Dosage

With respect to the therapeutic methods of the invention, it is not intended that the administration of the polypeptide or polynucleotide encoding such a protein be limited to a particular mode of administration, dosage, or frequency of dosing; the present invention contemplates all modes of administration, including intramuscular, intravenous, intraperitoneal, intravesicular, intraarticular, intralesional, subcutaneous, or any other route sufficient to provide a dose adequate to treat the P-gp-related disorder. The therapeutic may be administered to the patient in a single dose or in multiple doses. When multiple doses are administered, the doses may be separated from one another by, for example, one hour, three hours, six hours, eight hours, one day, two days, one week, two weeks, or one month. For example, the therapeutic may be administered for, e.g., 2, 3, 4, 5, 6, 7, 8, 10, 15, 20, or more weeks. It is to be understood that, for any particular subject, specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. For example, the dosage of the therapeutic can be increased if the lower dose does not provide sufficient therapeutic activity.

While the attending physician ultimately will decide the appropriate amount and dosage regimen, therapeutically effective amounts of the polypeptides of the invention protein may provided at a dose of 0.0001, 0.01, 0.01 0.1, 1, 5, 10, 25, 50, 100, 500, or 1,000 mg/kg.

Diseases

The polypeptides of the invention may be used to treat any disease where inhibition of the interaction between caveolin-1 and P-gp is desired or increased P-gp efflux is desired. As we have shown that the exemplary peptides pepCav

and pepPgp are capable of decreasing angiogenesis and cellular migration, the polypeptides of the invention may be used to treat any neoplasm such as cancer. Exemplary cancers include leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

In addition, neurological diseases, such as neurodegenerative diseases, may also be associated with decreased P-gp activity. Patient suffering from, or at increased risk of developing, a neurological disease (e.g., the neurodegenerative diseases described herein) may therefore benefit from

increased P-gp activity. Accordingly, the polypeptides of the invention may be used to treat patients suffering from diseases such as AD, PD, Huntington's disease, Prion disease, bovine spongiform encephalopathy, CJD, ALS, Lewy body disease, or Pick's disease.

Other diseases that can be treated with the polypeptides of the invention include drug intoxications (e.g., overdose), inclusion body myositis, cerebral amyloid angiopathy, amyloidosis (e.g., AA-type), and eye diseases (e.g., macular degeneration and glaucoma).

Formulation of pharmaceutical compositions

The administration of an polypeptide or polynucleotide may be by any suitable means that results in a concentration of the protein that treats the disorder. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for the oral, parenteral (e.g., intravenously or intramuscularly), intraperitoneal, rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular administration route. Thus, the composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: *The Science and Practice of Pharmacy*, 20th edition, 2000, ed. A.R. Gennaro, Lippincott Williams & Wilkins, Philadelphia, and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Pharmaceutical compositions according to the invention may be formulated to release the active compound immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create substantially constant concentrations of the agent(s) of the invention within the body over an extended period of time; (ii) formulations that after a predetermined lag time create substantially constant concentrations of the agent(s) of the invention within the body over an extended period of time; (iii) formulations that sustain the agent(s) action during a predetermined time period by maintaining a relatively constant, effective level of the agent(s) in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the agent(s) (sawtooth kinetic pattern); (iv) formulations that localize action of agent(s), e.g., spatial placement of a controlled release composition adjacent to or in the diseased tissue or organ; (v) formulations that achieve convenience of dosing, e.g., administering the composition once per week or once every two weeks; and (vi) formulations that target the action of the agent(s) by using carriers or chemical derivatives to deliver the therapeutic to a particular target cell type.

Administration of the protein in the form of a controlled release formulation is especially preferred for compounds having a narrow absorption window in the gastro-intestinal tract or a relatively short biological half-life.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the protein is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the protein in a

controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, molecular complexes, microspheres, nanoparticles, patches, and liposomes.

Parenteral compositions

The pharmaceutical composition may be administered parenterally by injection, infusion, or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation.

Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for implantation, or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active agent(s), the composition may include suitable parenterally acceptable carriers and/or excipients. The active agent(s) may be incorporated into microspheres, microcapsules, nanoparticles, liposomes, or the like for controlled release. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing agents.

As indicated above, the pharmaceutical compositions according to the invention may be in a form suitable for sterile injection. To prepare such a composition, the suitable active agent(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents

that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, dextrose solution, and isotonic sodium chloride solution. The aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

Controlled release parenteral compositions

Controlled release parenteral compositions may be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, or emulsions. The composition may also be incorporated in biocompatible carriers, liposomes, nanoparticles, implants, or infusion devices.

Materials for use in the preparation of microspheres and/or microcapsules are, e.g., biodegradable/bioerodible polymers such as polygalactin, poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutamnine), poly(lactic acid), polyglycolic acid, and mixtures thereof. Biocompatible carriers that may be used when formulating a controlled release parenteral formulation are carbohydrates (e.g., dextrans), proteins (e.g., albumin), lipoproteins, or antibodies. Materials for use in implants can be non-biodegradable (e.g., polydimethyl siloxane) or biodegradable (e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters)) or combinations thereof.

Solid dosage forms for oral use

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients, and such formulations are known to the skilled artisan (e.g., U.S.P.N.: 5,817,307, 5,824,300, 5,830,456, 5,846,526, 5,882,640, 5,910,304, 6,036,949, 6,036,949, 6,372,218, hereby incorporated by reference). These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and anti-adhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

The tablets may be uncoated or they may be coated by known techniques, optionally to delay disintegration and absorption in the gastrointestinal tract and thereby providing a sustained action over a longer period. The coating may be adapted to release the protein in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the agent(s) until after passage of the stomach (enteric coating). The coating may be a sugar coating, a film coating (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose,

hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or an enteric coating (e.g., based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose). Furthermore, a time delay material such as, e.g., glyceryl monostearate or glyceryl distearate, may be employed.

The solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes, (e.g., chemical degradation prior to the release of the active substances). The coating may be applied on the solid dosage form in a similar manner as that described in Encyclopedia of Pharmaceutical Technology, supra.

The compositions of the invention may be mixed together in the tablet, or may be partitioned. In one example, a first agent is contained on the inside of the tablet, and a second agent is on the outside, such that a substantial portion of the second agent is released prior to the release of the first agent.

Formulations for oral use may also be presented as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate, or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders and granulates may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus, or spray drying equipment.

Gene therapy

A polypeptide of the invention can be effectively administered to a cell or patient using gene therapy techniques. See, generally, for example, U.S. Pat.

No. 5,399,346. The general principle is to introduce the polynucleotide into a target cell in a patient, and allow it to express a polypeptide that enhances the activity of the endogenous P-gp protein.

Entry into the cell is facilitated by suitable techniques known in the art such as providing the polynucleotide in the form of a suitable vector, or encapsulation of the polynucleotide in a liposome.

A desired mode of gene therapy is to provide the polynucleotide in such a way that it will replicate inside the cell, enhancing and prolonging the desired effect. Thus, the polynucleotide is operably linked to a suitable promoter, such as the natural promoter of the corresponding gene, a heterologous promoter that is intrinsically active in liver, neuronal, bone, muscle, skin, joint, or cartilage cells, or a heterologous promoter that can be induced by a suitable agent.

Examples

The following examples illustrate potential applications of the invention and are not intended to limit scope. Modifications and variations may be made therein without departing from the spirit and scope of the invention.

Example 1

Stimulation of the P-gp efflux transport activity of vinblastine in vitro

The effect of compounds PepPgp and PepCav on P-gp efflux transport activity was studied in Madin-Darby canine kidney (MDCK) cells stably transfected with *MDR1*. Cells were incubated in the presence of pepPgp or pepCav compounds (20 μg/ml). At different times following treatment, the accumulation of vinblastine, a specific substrate of P-gp, was measured. Figure 1A shows that accumulation of [^{3}H]-vinblastine decreased as a function of time in the presence of both compounds. This result indicates that P-gp transport

activity is stimulated in MDCK-*MDR1* cells. Furthermore, the addition of increasing concentrations of compounds reduced [^{3}H]-vinblastine accumulation in MDCK-*MDR1* cells (Figure 1B). These results indicate that both exemplary compounds stimulate P-gp transport in a dose-dependent manner. These are the first results describing the action of stimulatory compounds on P-gp transport activity.

Example 2

Stimulation of the P-gp efflux transport activity of beta-amyloid in vitro

The effect of compounds on P-gp uptake of beta-amyloid is studied in MDCK cells. Cells are incubated with beta-amyloid (catalog number PRO-447 from ProSpec-Tany TechnoGene LTD), a specific substrate of P-gp. PepPgp or pepCav compounds (20 μg/ml) is then added. At different times of treatment, the accumulation of beta-amyloid is measured. A decrease in beta-amyloid uptake observed in the presence of either one of the compounds is indicative of their stimulatory effect on P-gp efflux transport activity.

Example 3

Stimulation of the P-gp efflux transport activity in vivo

The effect of compounds on P-gp efflux transport activity was investigated in vivo. For this purpose, the brain uptake of [^{3}H]-Taxol, a specific substrate of P-gp, was measured. [^{3}H]-Taxol was perfused into the brain through the right jugular vein for 5 minutes in the presence or absence of pepCav or pepPgp at a concentration of 20 μg/ml. Radioactivity of [^{3}H]-Taxol was analyzed in the total brain, the capillary and the parenchyma. As shown in Figure 2, Taxol passage was reduced in the total brain by 50% and 30%, respectively, for pepCav and pepPgp as compared to control mice. The reduction was similar in the capillaries and in the parenchyma, indicating that

the increased activity of P-gp reduced the accumulation of [^{3}H]-Taxol in endothelial cells as well as the brain uptake. These results indicate that the compounds are able to stimulate P-gp transport activity in vivo at the blood brain barrier.

Example 4

Inhibition of cellular migration

The effect of both compounds on cell migration, was determined by the migration of MDCK-*MDR1* cells using Transwell filters (Costar; 8 μm pore size) precoated with 0.15% gelatin. Briefly, 1 x 10^5 cells were resuspended in 100 μl of serum-free medium with or without compounds (20 μg/ml). The lower chamber of the Transwell contained 10% serum used as chemoattractant. Cell migration was determined using a Nikon TMS-F microscope and Northern Eclipse Software. As observed in Figures 3A and 3B, pepPgp and pepCav inhibited cells migration of MDCK-*MDR1* by 30% and 80%, respectively, as compared to the control.

Example 5

Inhibition of angiogenesis in vitro

The effect of these compounds on angiogenesis was evaluated in vitro using human umbilical vein endothelial cells (HUVEC) tube formation on Matrigel. Following transfections, cells were trypsinized and 2.5 x 10^4 cells were seeded on Matrigel. After cellular adhesion, the medium was removed and 100 μl fresh medium was added with or without 40 μg/ml of either pepCav or pepPgp. Tube formation was evaluated after 18 hours. After incubation, tubular structures were visualized at 60x magnification using a Nikon TMS-F microscope and Northern Eclipse Software. As shown in figure 4A, treatment

of HUVEC cells with pepCav and pepPgp reduced the capillary-like structure formation by 75% and 40%, respectively.

Example 6

Inhibition of angiogenesis in vivo

The effect of these compounds on angiogenesis was evaluated in vivo using the Matrigel plug assay. Briefly, Matrigel containing heparin and bFGF was injected under the skin of mice in the presence or absence of 100 μg/ml pepCav and pepPgp. The Matrigel plug was removed after one week. Hemoglobin content in the Matrigel plugs, which corresponds to the vessels formed, was measured. As indicated in Figure 4B, treatment with 40 μg/ml pepCav and pepPgp reduced the hemoglobin content by 25% and 40%, respectively, indicating the both compounds inhibit angiogenesis in vivo.

Other embodiments

All patents, patent applications including U.S. Provisional Application No. 60/852,678, filed October 19, 2006, and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent, patent application, or publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

CLAIMS

1. A polypeptide comprising an amino acid sequence having at least 70% identity to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR, wherein said polypeptide is less than 170 amino acids in length.

2. The polypeptide of claim 1, wherein said polypeptide is less than 50 amino acids in length.

3. The polypeptide of claim 1 comprising an amino acid sequence having at least 70% sequence identity to the sequence DGIWKASFTTFTVTKYWFYR, wherein the polypeptide includes a valine or leucine at a position corresponding to the thirteenth amino acid of the sequence, a tyrosine at the position corresponding to the sixteenth amino acid of the sequence, a lysine or arginine at the position corresponding to the twentieth amino acid of the sequence, or a combination thereof.

4. The polypeptide of claim 1 comprising an amino acid sequence having at least 70% sequence identity to the sequence VTKYWFYR, wherein the polypeptide includes a valine or leucine at the position corresponding to the first amino acid of the sequence, a tyrosine at the position corresponding to fourth amino acid of the sequence, a lysine or arginine at the position corresponding to the eighth amino acid of the sequence, or a combination thereof.

5. The polypeptide of claim 1, wherein said polypeptide has at least one amino acid substitution or deletion relative to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

6. The polypeptide of claim 1, wherein said amino acid sequence has at least 95% identity to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

7. The polypeptide of claim 1 comprising the amino acid sequence of DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

8. The polypeptide of claim 1 consisting of the amino acid sequence of DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

9. The polypeptide of claim 1, wherein said polypeptide is capable of interacting with P-glycoprotein.

10. The polypeptide of claim 1, wherein said polypeptide is soluble.

11. The polypeptide of claim 1, wherein said polypeptide is substantially pure.

12. A composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

13. A polypeptide comprising: (a) an amino acid sequence having at least 70% identity to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR, and (b) a heterologous sequence.

14. The polypeptide of claim 13, wherein said amino acid sequence of (a) is at least 95% identical to the sequence DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

15. The polypeptide of claim 13, wherein said amino acid sequence of (a) is DGIWKASFTTFTVTKYWFYR or VTKYWFYR.

16. The polypeptide of claim 13, wherein said polypeptide is substantially pure.

17. The polypeptide of claim 13, wherein said polypeptide is capable of interacting with P-glycoprotein.

18. A composition comprising the polypeptide of claim 13 and a pharmaceutically acceptable carrier.

19. A soluble polypeptide comprising a fragment of caveolin-1, wherein said fragment binds to a portion of P-glycoprotein.

20. The polypeptide of claim 19, wherein said portion includes amino acids 36-47 of P-glycoprotein.

21. A composition comprising the polypeptide of claim 19 and a pharmaceutically acceptable carrier.

22. A method of increasing P-gp-mediated efflux in a cell, said method comprising administering a polypeptide of claim 1, 13, or 19 in an amount sufficient to increase P-gp-mediated efflux.

23. The method of claim 22, wherein said cell is in a patient.

24. The method of claim 23, wherein said patient is suffering from a neoplasm, a neurological disease, drug intoxication, inclusion body myositis, cerebral amyloid angiopathy, amyloidosis, or an eye disease.

25. The method of claim 24, wherein said polypeptide is administered in an amount sufficient to treat said condition or disease.

26. The method of claim 24, wherein said neoplasm is cancer.

27. The method of claim 26, wherein said cancer is selected from the group consisting of leukemia, polycythemia vera, lymphoma, Waldenstrom's macroglobulinemia, heavy chain disease, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, glioblastoma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

28. The method of claim 24, wherein said neurological disease is a neurodegenerative disease.

29. The method of claim 28, wherein said neurodegenerative disease is Alzheimer's disease, Parkinson's disease, Huntington's disease, prion disease, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, Lewy body disease, or Pick's disease.

30. The method of claim 24, wherein said eye disease is macular degeneration or glaucoma.

31. A polypeptide comprising an amino acid sequence having at least 70% identity to the sequence VFSMFRYSNWLDK, wherein said polypeptide is less than 1000 amino acids in length.

32. The polypeptide of claim 31, wherein the polypeptide has a valine or leucine at the position corresponding to the first amino acid of the sequence, a tyrosine at the position corresponding to the seventh amino acid of the sequence, a lysine or arginine at the position corresponding to the thirteenth amino acid of the sequence, or a combination thereof.

33. The polypeptide of claim 31, wherein said polypeptide has at least one amino acid substitution or deletion relative to the sequence VFSMFRYSNWLDK.

34. The polypeptide of claim 31, wherein said polypeptide is less than 50 amino acids.

35. The polypeptide of claim 31, wherein said amino acid sequence has at least 95% identity to the sequence VFSMFRYSNWLDK.

36. The polypeptide of claim 31 comprising the amino acid sequence VFSMFRYSNWLDK.

37. The polypeptide of claim 31 consisting of the amino acid sequence of VFSMFRYSNWLDK.

38. The polypeptide of claim 31, wherein said polypeptide is capable of interacting with caveolin-1.

39. The polypeptide of claim 31, wherein said polypeptide is soluble.

40. The polypeptide of claim 31, wherein said polypeptide is substantially pure.

41. A composition comprising the polypeptide of claim 31 and a pharmaceutically acceptable carrier.

42. A polypeptide comprising: (a) an amino acid sequence having at least 70% identity to the sequence VFSMFRYSNWLDK, and (b) a heterologous sequence.

43. The polypeptide of claim 42, wherein said amino acid sequence of (a) is at least 95% identical to the sequence VFSMFRYSNWLDK.

44. The polypeptide of claim 42, wherein said amino acid sequence of (a) is VFSMFRYSNWLDK.

45. The polypeptide of claim 42, wherein said polypeptide is substantially pure.

46. A composition comprising the polypeptide of claim 42 and a pharmaceutically acceptable carrier.

47. The polypeptide of claim 42, wherein said polypeptide is capable of interacting with caveolin-1.

48. A soluble polypeptide comprising a fragment of P-glycoprotein, wherein said fragment binds to a portion of caveolin-1.

49. The polypeptide of claim 48, wherein said portion of caveolin-1 includes amino acids 82-101.

50. The polypeptide of claim 48, wherein said polypeptide is substantially pure.

51. A method of increasing P-gp-mediated efflux in a cell, said method comprising administering a polypeptide of claim 31, 42, and 48 in an amount sufficient to increase P-gp-mediated efflux.

52. The method of claim 51, wherein said cell is in a patient.

53. The method of claim 52, wherein said patient is suffering from a neoplasm, a neurological disease, drug intoxication, inclusion body myositis, cerebral amyloid angiopathy, amyloidosis, or an eye disease.

54. The method of claim 53, wherein said polypeptide is administered in an amount sufficient to treat said condition or disease.

55. The method of claim 54, wherein said neoplasm is cancer.

56. The method of claim 55, wherein said cancer is selected from the group consisting of leukemia, polycythemia vera, lymphoma, Waldenstrom's macroglobulinemia, heavy chain disease, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, glioblastoma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

57. The method of claim 54, wherein said neurological disease is a neurodegenerative disease.

58. The method of claim 58, wherein said neurodegenerative disease is Alzheimer's disease, Parkinson's disease, Huntington's disease, prion disease, bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, Lewy body disease, or Pick's disease.

59. The method of claim 54, wherein said eye disease is macular degeneration or glaucoma.

F

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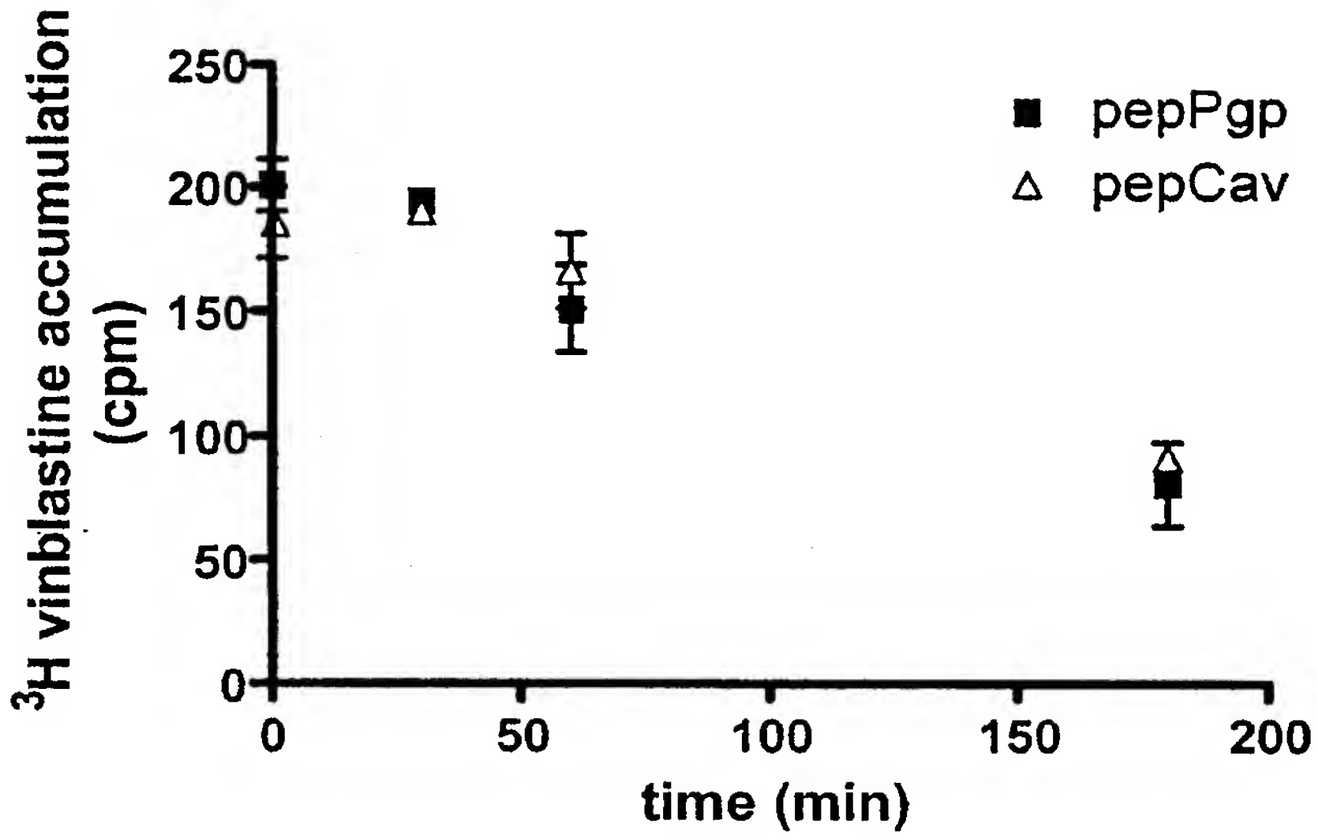
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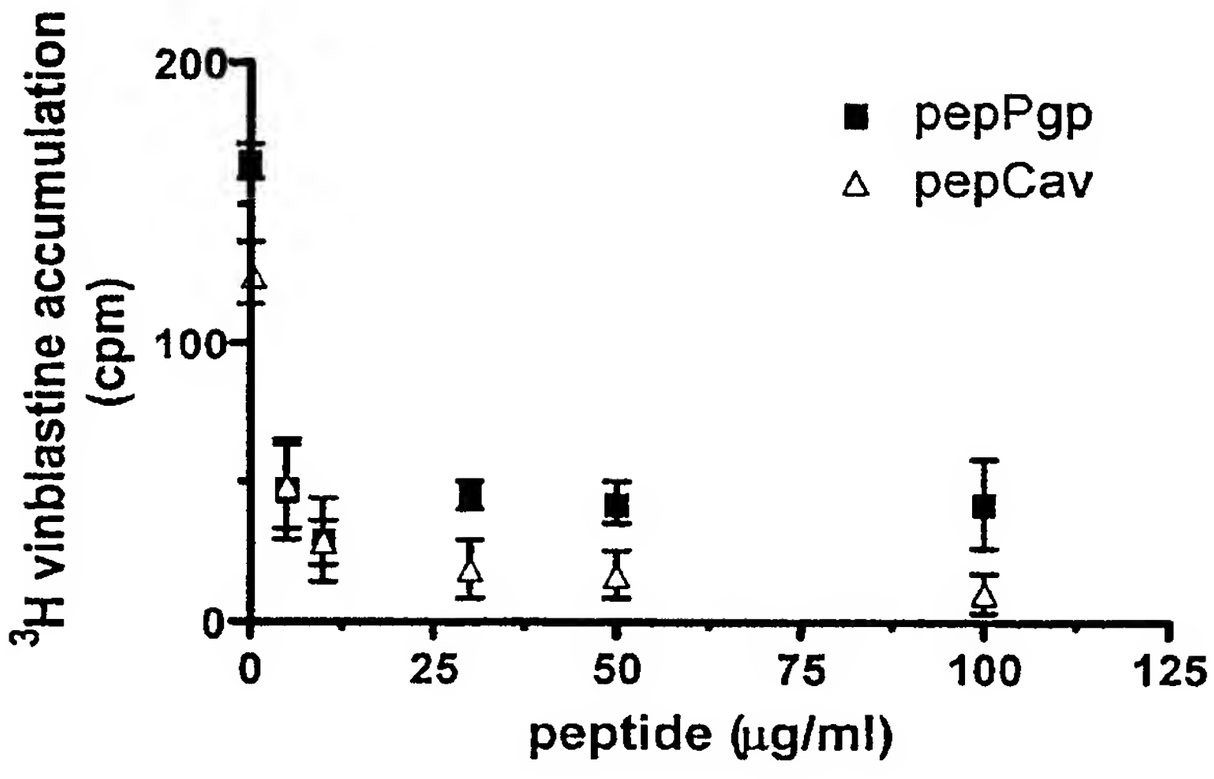
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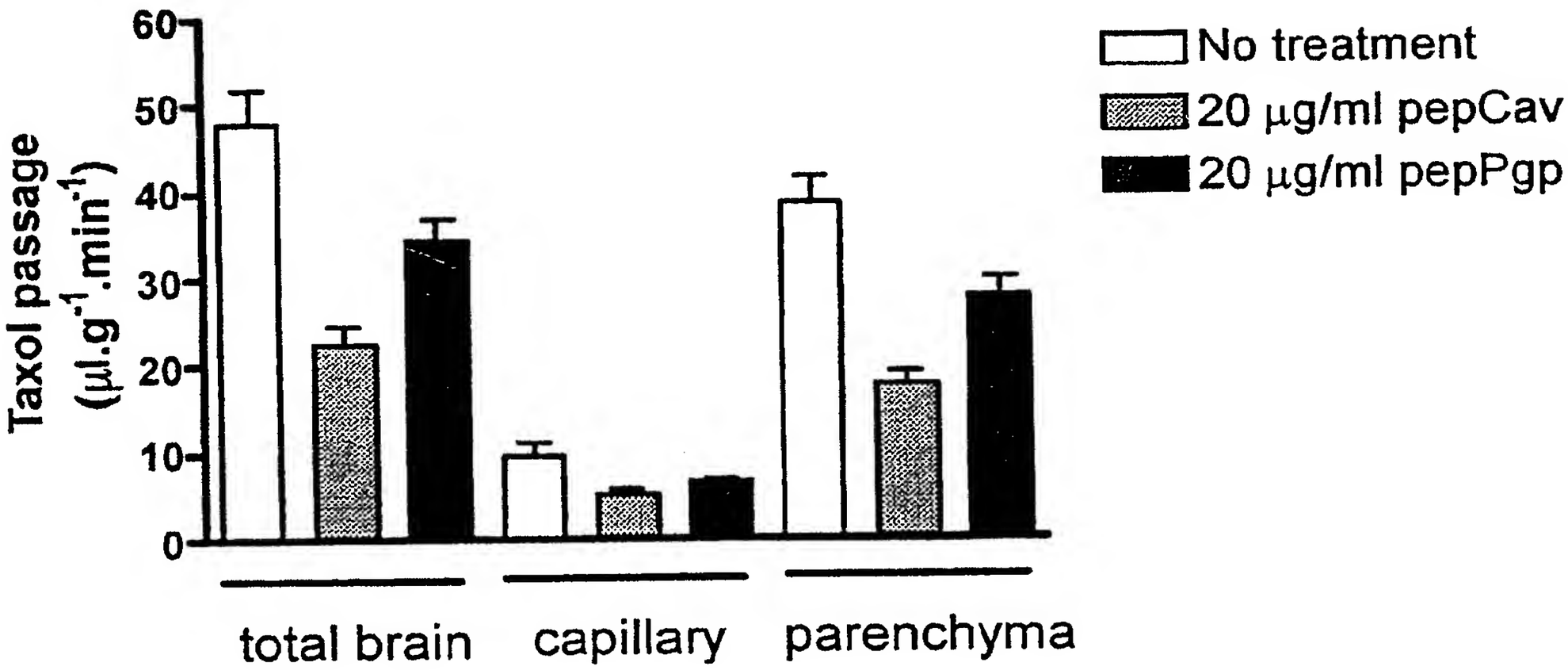
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F

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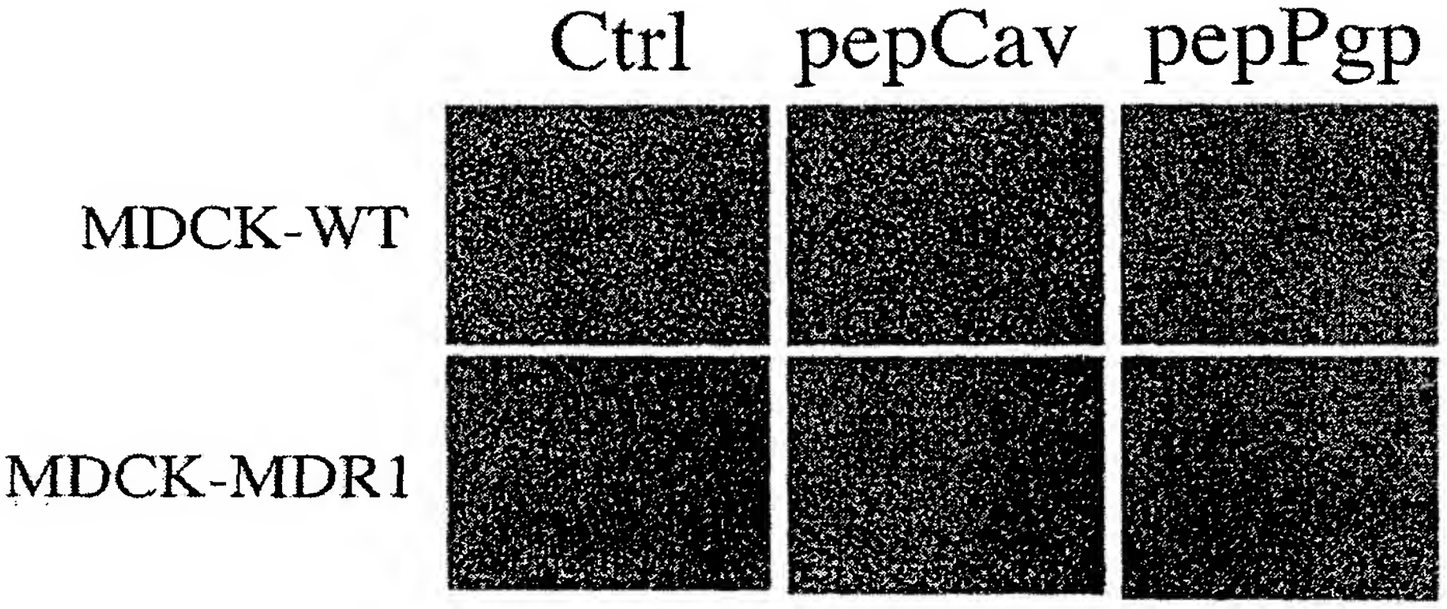
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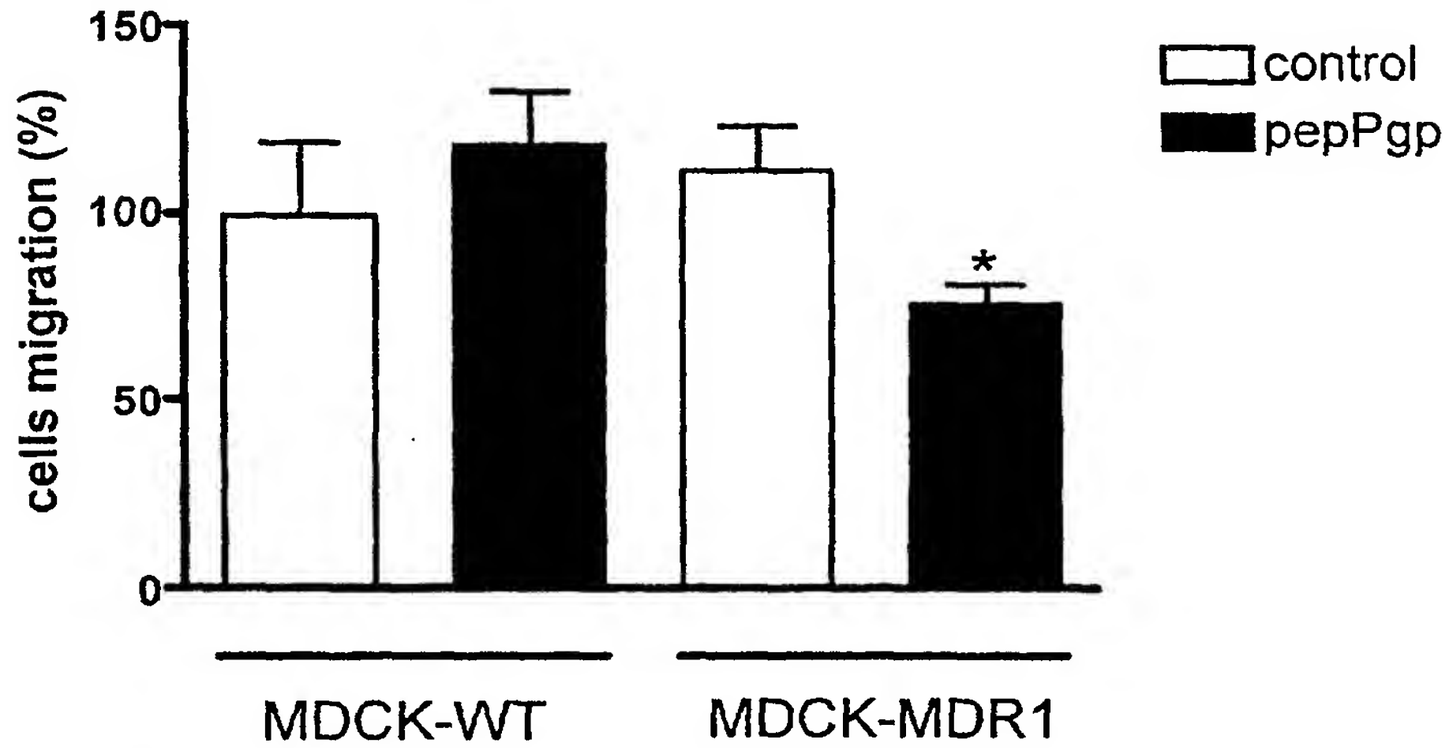
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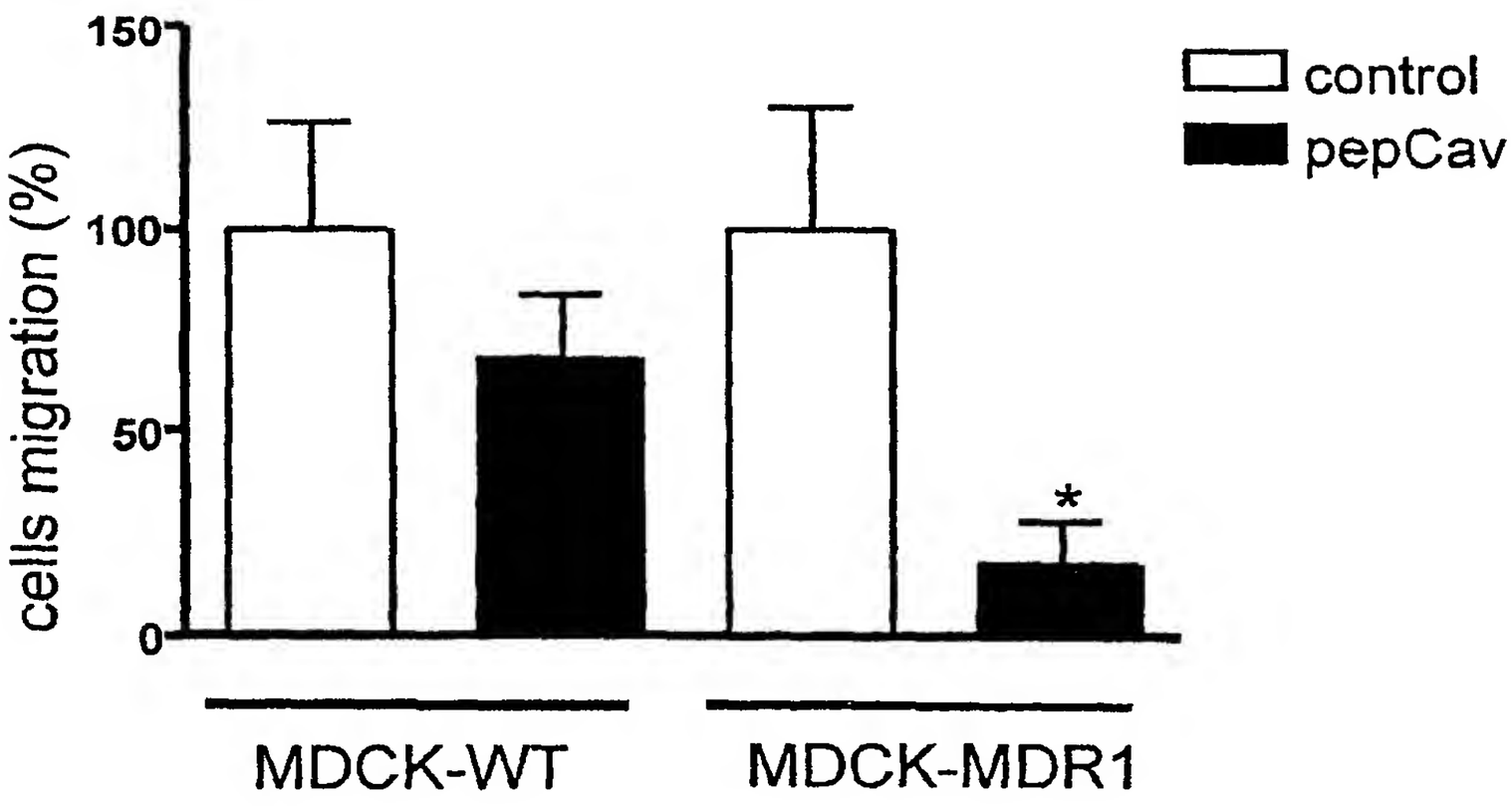
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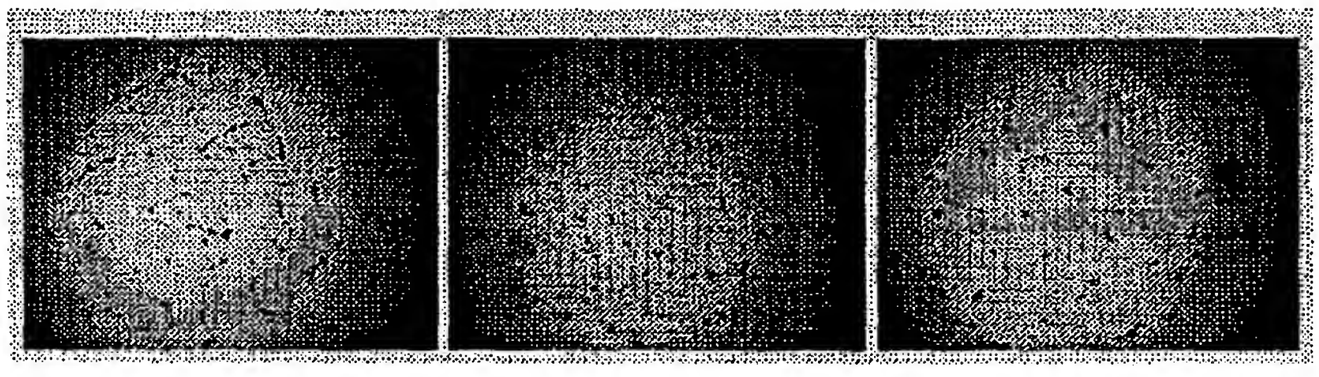
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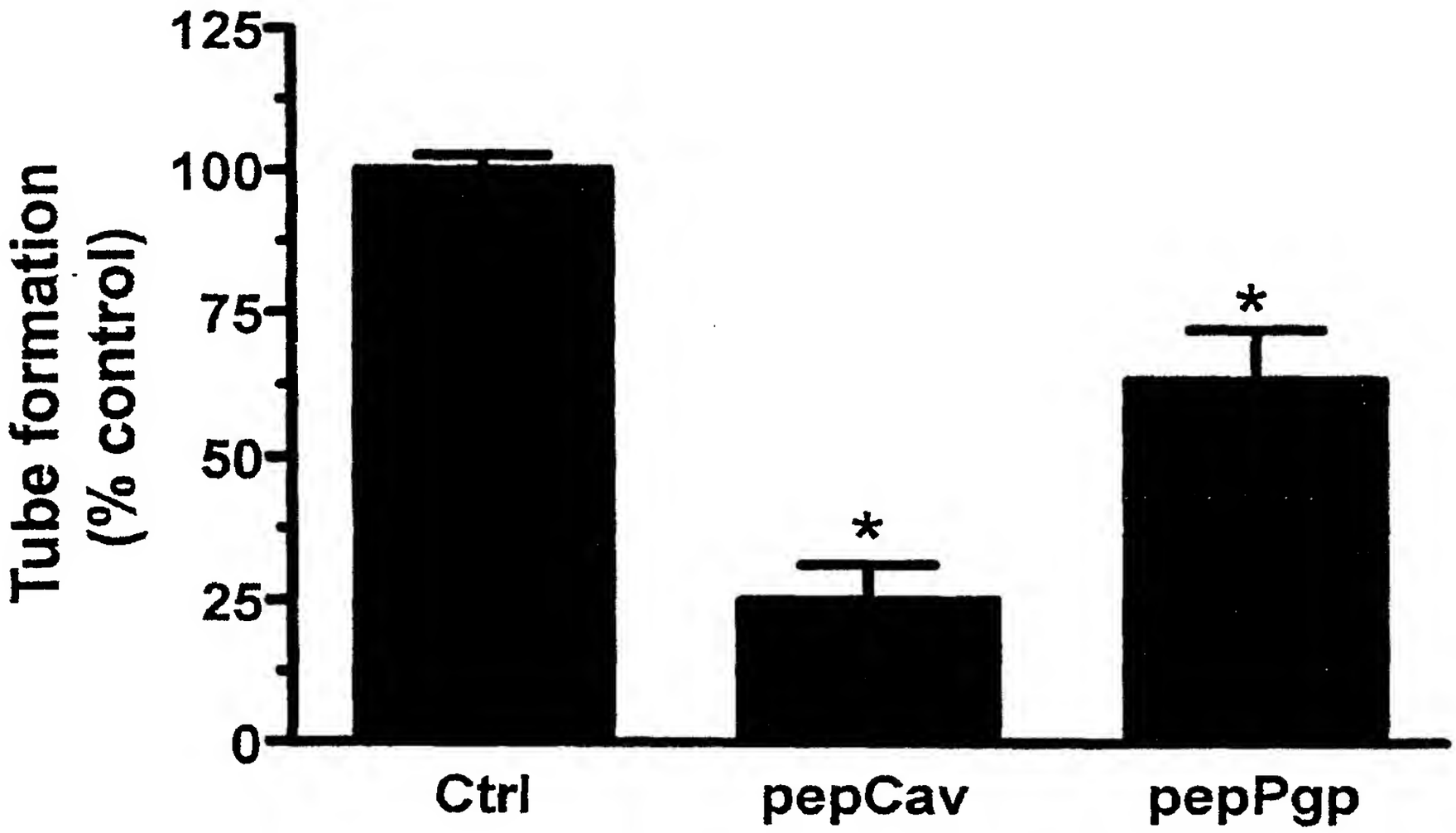
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*
*
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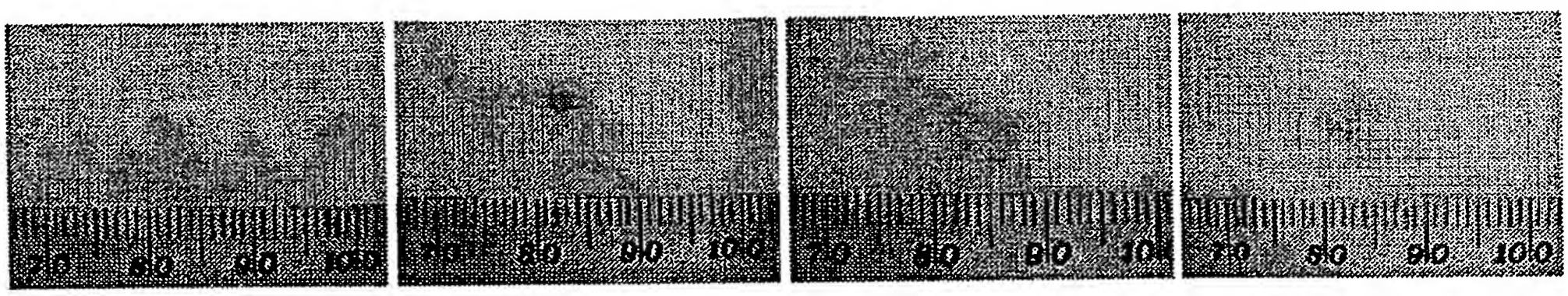
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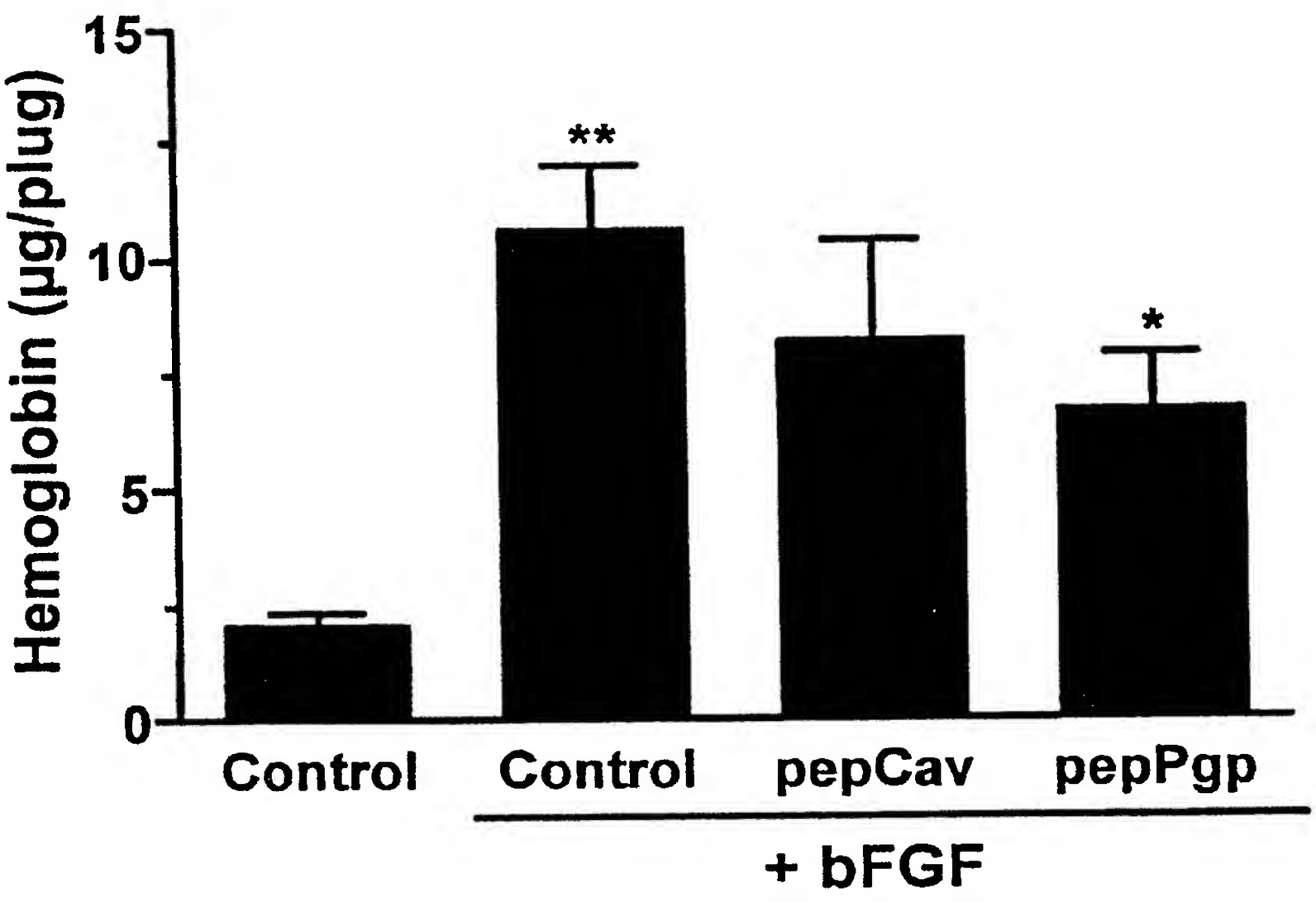
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*
Control
Control
pepCav
pepPgp
+ bFGF

Human P-glycoprotein

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  61 hgaglplmml vfgemtdifa nagnledlms nitnrsdind tgffmnleed mtryayyysg
 121 igagvlvaay iqvsfwclaa grqihkirkq ffhaimrqei gwfdvhdvge lntrltddvs
 181 kinegigdki gmffqsmatf ftgfivgftr gwkltlvila ispvlglsaa vwakilssft
 241 dkellayaka gavaeevlaa irtviafggq kkelerynkn leeakrigik kaitanisig
 301 aaflliyasy alafwygttl vlsgeysigq vltvffsvli gafsvgqasp sieafanarg
 361 aayeifkiid nkpsidsysk sghkpdnikg nlefrnvhfs ypsrkevkil kglnlkvqsg
 421 qtvalvgnsg cgksttvqlm qrlydptegm vsvdgqdirt invrflreii gvvsqepvlf
 481 attiaeniry grenvtmdei ekavkeanay dfimklphkf dtlvgergaq lsggqkqria
 541 iaralvrnpk illldeatsa ldteseavvq valdkarkgr ttiviahrls tvrnadviag
 601 fddgvivekg nhdelmkekg iyfklvtmqt agnevelena adeskseida lemssndsrs
 661 slirkrstrr svrgsqaqdr klstkealde sippvsfwri mklnltewpy fvvgvfcaii
 721 ngglqpafai ifskiigvft riddpetkrq nsnlfsllfl algiisfitf flqgftfgka
 781 geiltkrlry mvfrsmlrqd vswfddpknt tgalttrlan daaqvkgaig srlavitqni
 841 anlgtgiiis fiygwqltll llaivpiiai agvvemkmls gqalkdkkel egsgkiatea
 901 ienfrtvvsl tqeqkfehmy aqslqvpyrn slrkahifgi tfsftqammy fsyagcfrfg
 961 aylvahklms fedvllvfsa vvfgamavgq vssfapdyak akisaahiim iiektplids
1021 ysteglmpnt legnvtfgev vfnyptrpdi pvlqglslev kkgqtlalvg ssgcgkstvv
1081 qllerfydpl agkvlldgke ikrlnvqwlr ahlgivsqep ilfdcsiaen iaygdnsrvv
1141 sqeeivraak eanihafies lpnkystkvg dkgtqlsggq kqriaiaral vrqphillld
1201 eatsaldtes ekvvqealdk aregrtcivi ahrlstiqna dlivvfqngr vkehgthqql
1261 laqkgiyfsm vsvqagtkrq
```

Human Caveolin-1

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   1 msggkyvdse ghlytvpire qgniykpnnk amadelsekq vydahtkeid lvnrdpkhln
  61 ddvvkidfed viaepegths fdgiwkasft tftvtkywfy rllsalfgip maliwgiyfa
 121 ilsflhiwav vpciksflie iqcisrvysi yvhtvcdplf eavgkifsnv rinlqkei
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001861

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 14/705*** (2006.01) , ***A61K 38/17*** (2006.01) , ***A61P 25/28*** (2006.01) , ***A61P 27/06*** (2006.01) , ***A61P 35/00*** (2006.01) , ***C07K 14/47*** (2006.01), ***C07K 7/06*** (2006.01) , ***C07K 7/08*** (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K 14/705 (2006.01) , ***A61K 38/17*** (2006.01) , ***A61P 25/28*** (2006.01) , ***A61P 27/06*** (2006.01) , ***A61P 35/00*** (2006.01) , ***C07K 14/47*** (2006.01) , ***C07K 7/06*** (2006.01) , ***C07K 7/08*** (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Medline, CAPlus, Scopus, Delphion, PubMed; Sequence Search (Uniprot, RefSeq, NCBI GenPet, IPI, ENSEMBL, NCBI IGBlast, PDB, DrugBank) for DGIWKASFTTFTVTKYWFYR, VTKYWKY and VFSMFRYSNWLDK
Key words: p-glycoprotein, p-gp, inhibitors, caveolin, caveolin-1, caveolin scaffolding domain, C-20 peptide, neurological disease

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JODOIN et al. "P-glycoprotein in blood-brain barrier endothelial cells: interaction and oligomerization with caveolins", J. Neurochem. 2003, vol. 87(4), pages 1010-1023. ISSN 0022-3042 see whole document	19, 20, 21, 33, 48-50
Y		1-18, 22-30
X	DEMEULE et al. "Drug transport to the brain: Key roles for the efflux pump P-glycoprotein in the blood-brain barrier", Vascular Pharmacology, 2002, vol. 38, pages 339-348. ISSN 1570-1611 see Figure 2C, page 341	1-21
Y		22-30
P, X	BARAKAT et al. "Modulation of p-glycoprotein function by caveolin-1 phosphorylation", Journal of Neurochemistry, April 2007, vol. 101, pages 1-8. ISSN 0022-3042 see whole document	19-21
P, Y		1-18, 22-30

[X] Further documents are listed in the continuation of Box

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 2008 (07-01-2008)	4 February 2008 (04-02-2008)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Colleen MacFarlane 819- 997-4614

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001861

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 22-30 and 51-59

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 22-30 and 51-59 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under PCT Rule 39.1 (iv). Regardless, this Authority has carried out a search based on the alleged effects or purposes/uses of the products defined in claims 1-21

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

See extra sheet

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
X	OKAMOTO et al. "Caveolins, a Family of Scaffolding Proteins for Organizing 'Preassembled Signaling Complexes' at the Plasma Membrane", J. Biol. Chem., March 6, 1998, vol. 273(10), pages 5419-5422. ISSN 0021-9258 see whole document; Figure 1	1-21
Y		22-30
X	CA2421041 A (YALE UNIVERSITY), 14 March 2002 see whole document; page 12, lines 20-25; SEQ ID NO: 1; claims	1-21
Y		22-30
X	EP 1466924 A (INSTITUT PASTEUR; CENTRE NATIONAL DE LA RESCHERCHE SCIENTIFIQUE(CNRS)), 13 October 2004 see whole document; page 11, SEQ ID NO:10; Examples 14 and 15	1-21
Y		22-30
X	GB 2360453 A (UNIVERSITY COLLEGE CARDIFF CONSULTANTS LIMITED), 26 September 2001 see whole document; page 1, lines 24-30	1-21
Y		22-30
X	WO 99/46575 A (GEORGE-TOWN UNIVERSITY MEDICAL CENTER), 16 September 1999 see Table 1, page 12, SEQ ID NO:13	1-21
X	WO 97/40160 A (RIJKSUNIVERSITEIT TE GRONINGEN [NL/NL]), 30 October 1997 see Figure 1	31, 32, 36, 38-50
X	US 2002/0086384 A (LEVINE et al.), 4 July 2002 see page 101-102, SEQ ID NO: 67	31, 32, 36, 38-50

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2007/001861

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
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		EP1317487 A2	11-06-2003
		JP2005503321T T	03-02-2005
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		DE69933785T T2	04-10-2007
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		EP1717249 A1	02-11-2006
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		JP2003521223T T	15-07-2003
WO9740160	30-10-1997	AU2409397 A	12-11-1997
US2002086384	04-07-2002	IL136154D D0	20-05-2001

Continuation of Box III - Unity of Invention

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-30 are directed to polypeptides comprising fragments of caveolin-1, specifically DGIVKASFTTFTVTKYWFYR and VTKYWFYR, which are capable of binding to P-glycoprotein, and their use in the treatment of various conditions; and

Group B - Claims 31-59 are directed to polypeptides comprising fragments of P-glycoprotein, specifically VFSMFRYSNWLDK, which are capable of binding to caveolin-1, and their use in the treatment of various conditions.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

The claims of Groups A and B are not linked by a common inventive concept. While the desired result is the same, that is, an increase in P-glycoprotein-mediated efflux in a cell by the inhibition of caveolin-1 binding to P-glycoprotein, the mechanisms and compounds by which it is effected are entirely different. Group A claims are directed to polypeptides comprising fragments derived from caveolin-1 which bind to P-glycoprotein so as to competitively inhibit the binding of caveolin-1 itself to P-glycoprotein. Group B claims are directed to polypeptides of P-glycoprotein which bind caveolin-1, inhibiting it from binding to P-glycoprotein. The two groups use two different peptides in different ways to arrive at the desired result.